

USE OF PHOTOLUMINESCENT NANOPARTICLES FOR PHOTODYNAMIC THERAPY

RELATED APPLICATIONS

- Benefit of priority under 35 U.S.C. 119(e) is claimed to U.S. provisional application Serial No. 60/272,877, filed March 2, 2001, to Chen, entitled "NOVEL USES OF PHOTOLUMINESCENT
- 5 NANOPARTICLES FOR PHOTODYNAMIC THERAPY;" and to U.S. application Serial No. 09/798,277, filed March 2, 2001, and converted to a provisional application on February 26, 2002, serial number unavailable, to Chen, entitled "NOVEL USES OF PHOTOLUMINESCENT
- 10 NANOPARTICLES FOR PHOTODYNAMIC THERAPY." The disclosures of the above-referenced applications are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

- The invention relates generally to photodynamic therapy (PDT), and more specifically to illumination systems, compositions and methods of
- 15 using light-emitting nanoparticles to activate photosensitive compounds at the target site for PDT.

BACKGROUND

- PDT is a form of energy-activated therapy for destroying abnormal or diseased tissue. The procedure for this treatment includes
- 20 administration of a photosensitive compounds (a PDT drug) to a patient, followed by illumination with light having a wavelength or waveband corresponding to a characteristic absorption wavelength of the photosensitive compound. Upon illumination, the photosensitive compound absorbs photons from the light source and transfers this
- 25 energy to surrounding oxygen molecules. This in turn induces formation of singlet oxygen and other highly-reactive free radical species, leading to a number of biological effects, including damages to proteins, nucleic acids, lipids, and other cellular components, and often ultimately results in cell death.

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PDT drugs may be administered to a patient by ingestion or injection or by applying the compound to a specific treatment site on the patient's body. These compounds characteristically accumulate at higher concentrations in rapidly-growing tissue, such as malignant tumors, than in normal tissue. Typically, after administering a PDT drug intravenously and then waiting a period of time, the drug clears from normal tissues and is preferentially retained by rapidly-growing tissues. The drug remains inactive until exposed to light. Application of light of a suitable wavelength photoactivates the drug, resulting in generation of reactive species and damage to neighboring tissue. PDT has been used to treat various types of malignant tumors as well as non-cancerous condition such as macular degeneration and atherosclerosis.

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Light sources utilized for PDT include monochromatic lasers linked to fiber optics and light emitting diode (LED) arrays. One disadvantage of such light sources is that they are not capable of broadband emission at multiple wavelengths or wavebands at which a drug can be activated. Often a PDT drug can be activated at more than one wavelength. To obtain light of multiple wavelengths, light from multiple lasers and/or from multiple LEDs must be coupled into a fiber optic. Lasers can be bulky, requiring in-office or in-hospital administration of light and requiring a significant amount of valuable space to house the multiple lasers. Further LEDs may not provide all wavelengths desired. The technology to provide LEDs producing blue, violet, and ultraviolet light is developing, but LEDs are not yet available to provide the full spectrum of specific wavelengths that can be useful to active PDT drugs. Further, light in a waveband from the blue to ultraviolet part of the spectrum does not penetrate tissue very deeply. Consequently, any light administered via laser or LED array in this portion of the spectrum only penetrates shallow portions of tissue at the site where light is introduced to the body.

SUMMARY OF THE INVENTION

The invention provides a PDT therapy which is capable of utilizing light that deeply penetrates tissue, yet activates a wide range of PDT drugs, including those that activate at shorter wavelengths in which light

5 will penetrate only shallow portions of tissue. The invention also provides a PDT therapy in which multiple PDT drugs requiring light of diverse wavelengths can be used without having to resort to multiple lasers or LED arrays. In addition, the invention provides a surgeon with the capability to select from a wide array of PDT drugs, including those

10 whose use has been restricted or essentially prevented because of the short wavelength at which they absorb light.

Sub A4 The invention in one embodiment provides a photodynamic therapy in which light-emitting nanoparticles are administered to a patient in addition to a PDT drug in order to activate the drug. The light-emitting

15 nanoparticles absorb light from the light source and re-emit light at a different wavelength, one which is suitable to activate the PDT drug in the vicinity of the light emitting nanoparticles. The PDT drug near the light-emitting nanoparticles is activated, thus treating the disease anyplace that the PDT drug and nanoparticles are located and that light

20 from the light from the light source can reach.

The combination of light-emitting nanoparticles and PDT drug enables treatment of diseases using a broader array of PDT drugs and therapies than is presently available. For example, in another embodiment of the invention, a large tumor can be treated with a PDT

25 drug which is activated by a wavelength of light that penetrates shallowly into tissue (e.g. less than 1 cm). The light-emitting nanoparticles are selected so that they absorb light having a wavelength that penetrates deeply into tissue and then re-emit light at a wavelength that activates the PDT drug. Providing such nanoparticles in conjunction with the PDT

30 drug enables a significant portion of the depth of a large tumor to be

treated, whereas with the previously-known PDT therapy only the very surface of the tumor might be amenable to treatment.

The light-emitting nanoparticles administered with a PDT drug may be activated by single photon absorption of activating light. Alternatively, the light-emitting nanoparticles may be activated by two photon absorption of the activating light. Nanoparticles that absorb two photons often emit light at a wavelength that is about equal to or longer than the wavelength of the activating light, whereas nanoparticles that absorb a single photon typically emit light at a wavelength that is about equal to or shorter than the wavelength of the activating light. PDT drug-activating light of a desired wavelength, wavelengths, or waveband can therefore be provided by selecting the size and composition of the quantum dots as well as the wavelength or wavelengths of laser light used to illuminate the quantum dots.

A light-emitting nanoparticle that emits a wavelength useful in PDT can be modified to have one or more functional moieties. A functional moiety can provide better solubility in polar or aqueous materials or in organic materials. A functional moiety may also be a delivery moiety such as a particular protein, oligo- or polynucleotide sequence, antibody, or organism that place the light-emitting nanoparticle at or near the PDT treatment site.

Light-emitting nanoparticles can be used in any photodynamic therapy, not just tumor treatment. Consequently, light-emitting nanoparticles can be used in PDT to activate gene transcription, treat psoriasis, and treat macular degeneration among other diseases.

A light-emitting nanoparticle may be stimulated to emit light using light of a different wavelength that has been focused upon it by a total internal reflection lens. A total internal reflection lens concentrates light from a light source upon a desired area such as a tumor at which e.g. quantum dots or quantum rods are present, thus assuring that more of the light provided by the light source stimulates the nanoparticles to

fluoresce at the treatment site. Thus, any of the embodiments of the invention as described herein, including illumination systems and methods, may utilize or incorporate a total internal reflection lens as one of the elements focusing light from the light source.

- 5 Further, light from light-emitting nanoparticles may be focused upon the treatment site using a total internal reflection lens. Thus, the light emitted by such light-emitting nanoparticles as quantum dots or quantum rods is concentrated upon a desired area such as a tumor, thus assuring that more of the light emitted by the nanoparticles activates one
- 10 or more PDT drugs present at the treatment site. Thus, any of the embodiments of the invention as described here, including illumination systems and methods, may utilize or incorporate a total internal reflection lens as one of the elements focusing light from the light-emitting nanoparticles to the site to be treated, either with an additional total
- 15 internal reflection lens focusing light from the light source upon these nanoparticles or without the additional total internal reflection lens.

- The invention further provides an illumination system comprising a light source and light-emitting nanoparticles as well as an illumination system comprising a light source and a total internal reflection lens that is
- 20 configured to be positioned with the patient.

- Among other factors, the invention is based on the technical finding that a sufficient quantity of light-emitting nanoparticles in sufficient proximity to a PDT drug can activate the PDT drug, thereby generating a sufficient number of radicals such as singlet oxygen that a
- 25 therapy such as tumor treatment or gene transcription is effected. The light-emitting nanoparticles are generally much closer to critical areas within the site to be treated than conventional light sources can be positioned. Consequently, the depth of the site at which e.g. necrosis or transcription occurs beneath the skin is markedly greater than is possible
- 30 with conventional external PDT therapies. It is also possible to treat a greater thickness or depth of tumor or other tissue because light-emitting

nanoparticles can be distributed within the tissue to illuminate large portions of it and because activating light which penetrates tissue deeply but is otherwise poor at activating a PDT drug can be used to stimulate the nanoparticles to fluoresce. Light-emitting nanoparticles also allow a wider range of PDT drugs and therapies to be used, since the wavelength at which a PDT drug functions does not limit how the drug can be used. Further, light-emitting nanoparticles can activate a given PDT drug at multiple wavelengths simultaneously, imparting greater energy to the drug, and multiple drugs that activate at different wavelengths can be administered simultaneously when light-emitting nanoparticles are administered. Light-emitting nanoparticles having a delivery moiety attached or inserted at the treatment site also allow a PDT therapy to be focused or limited to the target site. Yet, tissue intermediate to e.g. a tumor and the surface of the skin is not affected by photodynamic therapy, even where the PDT drug is present in the intermediate tissue, where the wavelength of light absorbed by nanoparticles with delivery moieties does not activate the PDT drug. These technical findings and advantages and others are apparent from the discussion herein.

The invention thus provides a number of systems, components, compositions, means, and methods associated with photodynamic therapy and light-emitting nanoparticles as are more fully described below. This Summary section of the disclosure provides a summary of some salient points of the invention, but this section is not to be interpreted as limited the scope of the invention to only those features and embodiments discussed in this section. Instead, the invention involves all components, systems, and methods discussed in this and the following section that relate to compositions, systems, and methods involving light-emitting nanoparticles for PDT therapy in addition to those defined by the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates a scheme for attachment of a protein molecule to a nanoparticle.

FIG. 2 is a view of a tumor blood vessel, showing an antibody-linked nanoparticle in the lumen of the blood vessel, bound to an antigen on the interior of the surface vessel.

FIG. 3 illustrates light emitting nanoparticles with a biotin delivery moiety and a protocol for associating multiple nanoparticles with an individual cancer cell.

FIG. 4 depicts PDT treatment of tumor tissue using an external and/or interstitial light source to illuminate nanoparticles localized at the treatment site.

FIG. 5A illustrates insertion of an optical fiber composed of a biocompatible matrix with incorporated nanoparticles. An external LED or laser source provides light to the fiber.

FIG. 5B shows the optical fiber after use. The external portion of the fiber is cut off flush with the skin and buried.

FIG. 6A illustrates insertion of a polymeric sheath containing incorporated nanoparticles, using a removable needle.

Fig. 6B illustrates the sheath after the needle has been removed and a fiber optic light source inserted in its place.

Fig. 7A illustrates an optic fiber having a total internal reflection (TIR) lens at its end, inserted through a polymeric sheath in which are embedded nanoparticles. The polymer is preferably transparent to the light from the light source as well as the light emitted by the nanoparticles.

Fig. 7B depicts an optical fiber inserted into a polymeric sheath having a TIR lens at its end. The TIR lens captures light emitted by nanoparticles that would otherwise illuminate healthy tissue and directs it to diseases tissue.

Fig. 8A and 8B depict an optic fiber equipped with a TIR lens and having nanoparticles embedded in the end of the fiber (and/or coated onto the TIR lens along the optic path of the lens).

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

- 5 The invention provides a number of compositions, methods, and components in which light-emitting nanoparticles are used in photodynamic therapy. The following discussion provides further details on various aspects of the invention as well as discussion of certain preferred embodiments of the invention. The invention is, of course, not
- 10 limited to the preferred embodiments but it is instead to be accorded a scope consistent with the disclosed general principles as well as the various embodiments discussed herein. All patents and other references referred to herein are incorporated by reference in their entirety.

- The invention in one embodiment involves administering
- 15 therapeutically effective amounts of both photosensitive compound as used in photodynamic therapy and light-emitting nanoparticles to a patient, either separately or simultaneously. A light source producing light of a first wavelength illuminates the patient, externally or internally (or both). The light-emitting nanoparticles absorb this light and then emit
- 20 light of a second wavelength. The photosensitive compound absorbs light of the second wavelength and converts the energy imparted to the compound (a drug) into chemical energy by producing a reactive species such as singlet oxygen. The reactive species then either directly or
- 25 indirectly causes a desired event (such as cell necrosis or apoptosis or gene transcription).

 Prior to discussing therapies in which light-emitting nanoparticles are utilized, light-emitting nanoparticles and various forms they may take are described.

1. Light-emitting nanoparticles

There are a number of light-emitting nanoparticles that may be used, alone or in combination with one another, in photodynamic therapy. These include quantum dots, nanocrystals, and quantum rods.

1a) Quantum dots

5 *Sub Q5* Quantum dots are small molecular clusters having up to about a few hundred atoms. Quantum dots are therefore larger than individual atoms, but quantum dots generally behave in accord with the principles of quantum mechanics that govern the behavior of individual atoms.

10 Because of this behavior, quantum dots are sometimes also called "artificial atoms." Quantum dots have a size in the regime of about 1 nm to about 20 nm and are typically only a few nanometers in size.

Sub A6 15 A quantum dot is typically composed of a semiconductor material or materials, metal(s), or metal oxides exhibiting a certain bandgap energy. Although it is preferred that biocompatible light-emitting nanoparticles such as TiO_2 are used in the practice of the invention, nanoparticles that are not generally considered to be biocompatible may also be used. A variety of materials may be utilized for construction of nanoparticles, including but not limited to TiO_2 , Al_2O_3 , AgBr, CdSe, CdS, 20 $\text{CdS}_x\text{Se}_{1-x}$, CuCl, $\text{CdTe}_x\text{S}_{1-x}$, ZnTe, ZnSe, ZnS, GaN, InGaN, InP, CdS/HgS/CdS, and InAs/GaAs. Group II-VI, Groups III-V, and Groups I-VII semiconductors as well as Group IV metals and alloys form quantum dots and other nanoparticles as described below when formed sufficiently small. A quantum dot may also be surrounded by a material or materials 25 having wider bandgap energies (for example, ZnS-capped CdS), and especially may be surrounded by those materials that improve biocompatibility of the nanoparticles.

Quantum dots photoluminesce when stimulated by light having a wavelength in which the energy of a photon is at least equal to the 30 bandgap energy of the light-emitting material forming the quantum dot. Consequently, quantum dots absorb light of a first wavelength and emit

light at a second wavelength that is shorter than the first wavelength. The pump light supplied by e.g. a laser or light-emitting diode ("LED") array or other light source in which photons have an energy at least equal to the band-gap energy of the quantum dot is therefore absorbed by the quantum dot. The quantum dot re-emits energy in the form of light at a different wavelength and in a multidirectional fashion.

Because a quantum dot is so small and is thus governed by the rules of quantum mechanics, the size of the dot dictates the wavelength, and hence the color, of its fluorescence. The larger the diameter or width of a given quantum dot, the longer the wavelength of light that the quantum dot emits. For example, the band gap of a CdSe quantum dot can be tuned from deep red (1.7 eV) to green (2.4 eV) by reducing the diameter of the quantum dot from 200Å to 20Å.

There are a number of methods of making quantum dots. The synthesis of small semiconductor clusters in trioctylphosphine oxide (TOPO) at 300°C has been shown to yield highly fluorescent (quantum yields >50%) small particles of a number of semiconductor materials, such as CdSe, InP and InAs. Growth conditions such as the length of time of crystallization, concentration of monomer, and temperature establish the size of the quantum dot and therefore the color of the light emitted from the quantum dot. Such methods are discussed in the article by M. Green and P. O'Brien, "Recent advances in the preparation of semiconductors as isolated nanometric particles: new routes to quantum dots," *Chem. Commun.*, 1999, 2235-41 ("Green et al. article"). See also U.S. Pat. No. 5,909,670, 5,943,354, and 5,882,779.

1b) Nanocrystals

A nanocrystal is a molecular cluster of quantum dots that is large enough to have an identifiable interior, structurally identical to the corresponding bulk solid but having a substantial fraction of the total number of atoms on the surface. A nanocrystal has a size from about 20 nm to a size in which quantum effects no longer predominate (that is,

where the wavelength of light emitted is not a function of the diameter or size of the particle). Nanocrystals are generally spherical or are irregularly shaped. Nanocrystals may also be formed as disclosed in the Green et al. article as well as the patents cited above for quantum dots and other

5 methods known in the art.

1c) Quantum rods

A quantum rod is a structure formed of quantum dots in which the diameter or width of the rod exhibits quantum effects (that is, the wavelength of light emitted is a function of the diameter or width), but
 10 the rod has a length that is greater than that which contributes to the quantum effect (i.e., where the length has no effect on the wavelength of light emitted). Quantum rods emit much brighter light than individual quantum dots or nanocrystals formed of the same material.

Quantum rods can be made by controlling the growth kinetics of
 15 nanocrystals. One method of making quantum rods involves using both a very high concentration of monomer during crystal growth and a surfactant that allows for a controlled growth rate of crystalline structure. The article by X. Peng, L. Manna, W. Yang, J. Wickham, E. Scher, A. Kavanich & A. Alivisatos, "Shape control of CdSe nanocrystals," *Nature*,
 20 V. 404, Mar. 2, 2000, pp. 59 et seq. ("Peng et al. Article") discloses one such method.

1d) Superlattice

A three-dimensional array of quantum dots may be formed and used in accordance with the principles of the invention. For example,
 25 colloidal CdSe nanocrystals that are passivated with organic surfactants and size-selected to a very high degree so that they are of essentially uniform size will spontaneously precipitate from solution to form the array. The array can have bands of electronic states, as described in the article by A. Alivisatos, "Semiconductor Clusters, Nanocrystals, and
 30 Quantum Dots," *Science*, V 271, Feb. 16, 1996, 933-37 and the article

by C. Murray, C. Kagan, & M. Bawendi, *Science*, V.270, 1995, pp. 1335 et seq.

1e) Mixtures

Mixtures of quantum dots, nanocrystals, and/or quantum rods can
 5 be formed to match specific applications. For example, quantum rods of various sizes and compositions may be mixed to provide light of different wavelengths. The quantum rods of the mixture are selected based on their capacity to activate one or more selected photoactive compounds. For example, a number of PDT drugs absorb light and activate at two or
 10 more wavelengths. Consequently, quantum rods that emit those wavelengths can be mixed together and used to activate a PDT drug. These quantum rods can be formed of the same material (e.g. CdSe) but have different particle sizes that are selected to provide the appropriate wavelengths, or these quantum rods may be formed of different materials
 15 and have individual particle sizes that produce the wavelengths that activate the drug or drugs.

If desired, quantum rods of different diameter and optionally different lengths formed of the same material may be mixed together to provide light within a desired waveband or range of wavelengths, or
 20 quantum rods of approximately the same size but made of different materials can be mixed to provide the same waveband of light. A mixture of quantum rods that provides one or more wavebands of light is also useful to accommodate small variations in activation wavelength for a particular drug, where the photoactivation wavelength varies slightly from
 25 one molecule of the drug to the next molecule of that drug.

Quantum dots may be mixed with nanocrystals and/or quantum rods to provide a composition that emits light of multiple selected wavelengths, with the intensity of light emitted at one wavelength differing from the intensity of light emitted at a second wavelength. In
 30 this instance, nanocrystals would usually produce light of greater intensity than light produced by quantum dots, and quantum rods would

usually produce light of greater intensity than light produced by nanocrystals formed of the same light-emitting material.

PDT drugs are often subject to "bleaching," a phenomenon in which the drugs lose their effectiveness as a function of the light intensity used to activate the drug and the number of activation "cycles" that the drug experiences. Different drugs have different bleaching rates, and thus if a plurality of PDT drugs are used in combination in photodynamic therapy, different intensities of light at the various wavelengths may help to prevent premature bleaching. Further, even though a particular drug activates at multiple wavelengths, the amount of light absorbed at one wavelength usually differs from the amount of light that is absorbed at another wavelength for this drug. Consequently, it can be useful to provide a mixture of nanoparticles that provide a waveband of light in which the intensities of certain wavelengths within the waveband differ based on the drug or drugs to be employed in the photodynamic therapy.

The amount of light generated at a given wavelength can be selected by adjusting the length of a quantum rod. A quantum rod equal in diameter and material of construction to another quantum rod but made longer than the other quantum rod will produce a greater illuminance (flux per illuminated unit area) and fluence (time integrated flux of photons per illuminated area) of light. Therefore, a mixture of quantum rods of various lengths and diameters can be used to provide a light illuminance or fluence that varies by wavelength within the waveband generated by the mixture. Further, quantum rods can be mixed with quantum dots and/or nanocrystals to vary the illuminance or fluence of light as a function of the wavelength emitted.

A mixture of quantum dots, nanocrystals, and/or quantum rods may be selected to provide certain desired wavelengths of light, or the mixture may be selected to provide a substantially continuous spectrum of light between two wavelengths. Further, the mixture can be selected

to provide plural wavebands of emitted light. For example, a first light-emitting material is used to form nanoparticles (quantum dots, nanocrystals, and/or quantum rods), and the size of the particles is selected to provide light of a continuous or discrete spectrum of

- 5 approximately 20 nm around a second wavelength at which either the same drug or a different PDT drug activates. The two sets of nanoparticles are mixed to form the mixture. This mixture of nanoparticles thus emits light having wavelengths within two wavebands that are centered about the wavelengths at which a PDT drug or drugs
10 activate.

- The light-emitting nanoparticles of the mixture can also be selected so that they absorb light of a single wavelength or narrow waveband, as is produced by a laser, or the nanoparticles can be selected so that they absorb different wavelengths of light as supplied by a light source that
15 produces a wider range of wavelengths, such as that light supplied by an LED array. Thus, a mixture of particles can absorb light emitted within a narrow waveband and emit light within a narrow waveband, a broad waveband, or within multiple wavebands by selecting the materials forming the individual nanoparticles, their sizes, and/or their shapes as
20 described above.

- The various quantum dots, nanocrystals, and/or quantum rods may be mixed dry or in a liquid or gaseous carrier using methods known in the art for mixing small particles. Such carriers include blood, a blood product such as plasma, an oil, an ointment, a cream, or a gel.

25 2. Light-emitting nanoparticle having a group to solubilize the nanoparticle

- The light-emitting nanoparticles discussed above may have hydrophilic groups attached to them in order to render the nanoparticles soluble in polar solvents. One such procedure is disclosed in Chan and
30 Nie, Science 281, p. 2016 (1998) ("Chan et al. Article"). This procedure involves use of mercaptoacetic acid to solubilize ZnS-capped quantum

dots. When the reaction is performed in chloroform, the mercapto groups bind to the Zn atoms, and the free polar carboxylic acid groups render the quantum dots water-soluble.

Instead of using mercaptoacetic acid in a reaction with light-

- 5 emitting nanoparticles, a mercapto-organic material can instead be used to improve the solubility of light-emitting nanoparticles in organic liquids. Likewise, an amphipathic material, which improves nanoparticle solubility in either a polar or non-polar solvent, can be attached to a light-emitting nanoparticle through a mercapto group or other reactive group.

10 3. Light-emitting nanoparticle having a delivery moiety

- A light-emitting nanoparticle may be attached to a delivery moiety, which delivers the nanoparticle to a specific target site within the patient. A delivery moiety may be a protein, an oligo- or polynucleic acid, an antibody, a drug, an enzyme, an ion of high affinity for the target site, or
 15 other moiety that binds or complexes with the target site or a component found at the target site. Fig. 1 illustrates this type of nanoparticle **100**, in which a DdSe quantum dot **101** is coated with ZnS **102**, which has a wider or larger bandgap than CdSe has, and which is attached to protein **103** through a mercapto-acetic acid group that has reacted with a free
 20 amine on the protein. Especially preferred delivery moieties are antibodies that are specific to a site to be treated with PDT. For example, Fig. 2 illustrates a quantum dot **201** attached to an antibody **202** that binds to a blood vessel antigen **203** produced at a blood vessel **204** within a tumor. The antibody binds to the antigen, positioning the quantum dot or dots
 25 attached to the antibody at the site to be treated.

- Examples of delivery moieties include endogenous inhibitors of angiogenesis such as angiostatin (Entremed, Rockville, MD), Troponin-1 (Boston Life Sciences, Boston, MA), endostatin, TNP-470 (6-O-chloroacetylcarbonyl)-fumagillol (TOP Pharmaceuticals, Deerfield, IL), 2-
 30 methoxyestradiol, troponin-1, antithrombin, thrombospondin, metallospandins such as the proteins produced by the genes METH-1 and

METH-2, tyrosine kinases such as Tie-1 and Tie-2, TNP-470 (a synthetic analog of the antibiotic fumagillin), squalamine (Magainin Pharmaceuticals, Plymouth Meeting, PA), indirect angiogenesis inhibitors such as IM-862 (a two amino-acid synthetic version of a natural peptide

5 that upregulates interleukin-12 and inhibits vascular endothelial growth factor and basic fibroblast growth factor expression, Cytran, Kirkland, WA), interferon- α , interleukin-12 (Roche, Nutley, NJ), and pentosan polysulfate (Georgetown University, Washington, DC), combretastatin A-4 (OXiGENE, Boston, MA) thalidomide (Celgene, Warren, NJ), ZD-0101

10 (AstraZeneca, London, UK), therapeutics that target integrins such as $\alpha_v\beta_3$ and $\alpha_v\beta_5$ antagonists, antagonists that bind the angiogenic stimulator VEGF (such as those developed by Genentech, S. San Francisco, CA), other small molecules that block growth factor receptors such as Angiozyme (Ribozyme, Boulder, CO), PTK-787 and ZK-225846 (Novartis, Basil, Switzerland), and SU-5416, SU-101, and SU-6668 (Sugen, S. San

15 Francisco, CA), proteolysis inhibitors such as Neovastat (Aeterna, Ste. Foy, Quebec), Metastat (Collagenex, Newtown, PA), Marimastat (British Biotech, Oxford, UK), CGS-27023A (Novartis, E. Hanover, NJ), BMS-275291 (Bristol-Myers Squibb, Princeton, NJ), Bay-12-9566 (Bayer, West

20 Haven, CT), AG-3340 (Agouron, La Jolla, CA), $\alpha_v\beta_3$ trichosanthin, molecules that block EC-ECM adhesion such as EMD-121974 (Merck KcgaA, Darmstadt, Germany) and Vitaxin (Ixsys, La Jolla, CA).

Fig. 3 illustrates how many light-emitting nanoparticles may be targeted to each cancer cell 301 without associating the nanoparticles

25 with normal cells 303 by targeting the nanoparticles to antigens 302 on the cancer cells. An antibody 304 carrying multiple biotin molecules 305 is administered to the patient, and subsequently avidin or streptavidin 306 is administered to bind the biotin molecules carried by the antibody. Once the avidin or streptavidin molecule is bound to the biotinylated

30 antibody, light-emitting nanoparticles 307 having one or more biotin molecule delivery groups 308 attached are administered. Each avidin or

streptavidin molecule can bind multiple biotinylated light-emitting nanoparticles. Consequently, a plurality of light-emitting nanoparticles can be associated with each cancer cell.

- Other biotinylated site-specific materials such as monoclonal
- 5 antibodies, polyclonal antibodies, proteins, and oligo- or polynucleotides such as Fab fragments or proteins can be used in place of the antibody which binds to a tumor antigen in order to provide a wide range of target-specific components. Consequently, one member of an affinity binding set can be attached to a nanoparticle, and the other member of the
- 10 affinity binding set may be a site-specific component. Affinity binding sets include: biotin-avidin; biotin-streptavidin; antibody-antigen; antibody fragment-antigen; chemokine-chemokine receptor; and growth factor-growth factor receptor.

- The Chan et al. Article discusses a way of attaching quantum dots
- 15 having free carboxylic acid groups to a molecule such as a protein. In this method, a carboxylic acid group on a quantum dot reacts with a free amine group to form an amide or peptide group. Nanocrystals and quantum rods may likewise be attached to a protein or other target-specific component using this method.

- 20 Another method of attaching quantum dots or other nanoparticles to a delivery moiety is disclosed in U.S. Pat. 5,990,479 ('479 patent). The method of the '479 patent involves coating the nanoparticle with e.g. a thin coating (0.5 to 2 nm) of silica glass and linking the nanoparticle to a delivery moiety. The glass is linked to the nanoparticle
- 25 through a linking agent such as substituted silane (e.g. 3-mercaptopropyl-trimethoxysilane), and the delivery moiety is linked to the glass through a second linking agent such as an amine (e.g. 3-aminopropyl-trimethoxysilane). Other methods of attaching a nanoparticle to a delivery moiety are discussed in Z. Chunyang et al., "Quantum Dot-
- 30 Labelled Trichosanthin", *Analyst*, 2000, **125**, pp. 1029-1031 (quantum dot-labelled trichosanthin); L. Cattell et al., "The role of conjugation

processes and linking agents in the preparation of molecular/particulate conjugates - a review," *S.T.P. Pharma Sciences* 9 (4), pp. 307-319 (1999); WO 0029617; WO 0027365; and U.S. Pat. No. 5,055,446 and 5,985,353.

- 5 Coating light-emitting nanoparticles with silica or another material transparent to the wavelengths of light absorbed and emitted by the nanoparticles and that does not react to a significant extent with bodily fluids provides a way to improve biocompatibility of the nanoparticles. Consequently, nanoparticles that might otherwise not be considered
- 10 suitable for use in a human can be coated to improve their biocompatibility.

4. Light-emitting nanoparticle linked to PDT drug

- Light-emitting nanoparticles may be linked to a reactive group on a PDT drug by any of the methods described previously. Consequently,
- 15 light-emitting nanoparticles may be covalently bound or may be complexed with the drug through a delivery moiety. The light-emitting nanoparticles are therefore positioned sufficiently closely to the PDT drug to illuminate it, even in instances where the tissue penetration depth of the light emitted by the nanoparticles is very small. The number of
- 20 nanoparticles attached to the drug can also be selected to provide a sufficiently high concentration of nanoparticles around the PDT drug to assure the drug receives the intensity of light needed to activate the drug to provide the desired therapy.

- Further, both the PDT drug and the light-emitting nanoparticles
- 25 may be linked to an oligomeric or polymeric backbone. A polymer having free amine groups such as a melamine formaldehyde resin will react with both a PDT drug and nanoparticles that have been functionalized with carboxylic acid groups, and the PDT drug and nanoparticles can be spaced sufficiently closely to one another along the polymer that the
- 30 nanoparticles assure that the PDT drug is illuminated.

5. Encapsulated light-emitting nanoparticle

An alternate procedure for delivering nanoparticles to the treatment site involves encapsulating them in liposomes, microcapsules or nanocapsules, or other such drug carriers. Methods known to those in this field for incorporating small particles of a size similar to that of light-emitting nanoparticles can be utilized (see, e.g.; U.S. Pat. No. 5,686,113). Nanocapsules may be targeted to the treatment site by attachment of functional or delivery moieties on their outer surfaces as described above, such as antibodies or other delivery moieties. See also L. Cattell et al., "The role of conjugation processes and linking agents in the preparation of molecular/particulate conjugates - a review, " *S.T.P. Pharma Sciences* 9 (4), pp. 307-319 (1999).

6. Carrier for light-emitting nanoparticles

a) Liquid, emulsion, gel, or other fluid containing nanoparticles

Any of the components discussed above (e.g. light-emitting nanoparticles, light-emitting nanoparticles having a solubilizing group, light-emitting nanoparticles having a delivery moiety, and encapsulated light-emitting nanoparticles) can be placed in a carrier that is suitable for delivery to the site to be treated with PDT. Consequently, these components may be formulated with a sterile or biocompatible carrier suitable for introduction into a human such as saline, castor oil, blood plasma or plasma-containing liquids, purified water, or other liquid carrier such as a bodily fluid that is present in the area to be treated. Topical carriers include ointments, creams, or gels that may be applied to the skin.

Other compositions containing the above-discussed components may be formulated to include drugs such as one or more PDT drugs, antibiotics, heparin, chemotherapy agents, therapeutic radionuclides, and other drugs whose efficacy is not substantially diminished by the

presence of the light-emitting nanoparticles and which do not interfere substantially with light emission by the nanoparticles.

b. Immobilized light-emitting nanoparticles

Immobilized light-emitting nanoparticles can be introduced to an area by applying them topically or by inserting or implanting the immobilized nanoparticles into the body through an incision or via injection or by another known method. Immobilized light-emitting nanoparticles are not free to move on or within the body when first introduced, as the other forms of nanoparticles described above are able to move when first introduced into or on the body. Immobilized in or on a matrix formed of a grid, fiber, fabric or other substrate that does not move when applied on or in the body. The matrix may be solid but transparent to the wavelengths at which the nanoparticles absorb and emit light, or the matrix may be porous so that nanoparticles are embedded within pores that transport light to and from the nanoparticles. The matrix also may be solid and translucent or opaque to the wavelengths at which the nanoparticles absorb and emit light where the nanoparticles are placed on the surface of the matrix. Shapes and forms for the matrix used to carry the immobilized nanoparticles include but are not limited to a fibrous shape, a rod-like or cylindrical shape, a grid, or a film.

The PDT drug may also form part of the matrix. The PDT drug may be absorbed into the matrix, or molecules of the PDT drug may be attached to the surface of the matrix so that the drug and nanoparticles are administered simultaneously.

One example of the invention is shown in FIG. 5A and 5B. An optical fiber 501 composed of a biocompatible matrix is shown. Quantum dots 502 coat the external shell of the fiber or are embedded in the matrix. The fiber is inserted beneath a patient's skin 503 in proximity to the PDT treatment site. An external light source, such as an LED, a laser, a laser diode, or other non-coherent light source such as a

incandescent lamp or halogen light, pumps light into the fiber during treatment. After the treatment is complete, the externally exiting portion of the fiber is cut off flush with the skin **503** and buried, as illustrated in FIG. 5B. The fiber degrades over time and is reabsorbed. The optical

5 fiber can be formed of a polylactide as known in the art, for example.

In another example of the invention as illustrated in Fig. 6A and 6B, light-emitting nanoparticles **601** are incorporated into a polymeric sheath **602** for insertion beneath a patient's skin **603** in proximity to the treatment site **604**. A removable needle **605** is optionally used to stiffen

10 the sheath and to aid inserting the sheath into the site, as illustrated in Fig. 6A. The needle punctures the tissue to aid in positioning the sheath in the body, and the needle may also be used to inject the PDT drug if desired and optionally additional liquid-borne light-emitting nanoparticles into the treatment area. After the needle is withdrawn, leaving the

15 sheath embedded in the target site, a light source such as an optical fiber **606** may be inserted into the sheath and used to activate the nanoparticles in the sheath and the optional liquid-borne nanoparticles, which in turn activate the PDT drugs at the treatment site as shown in Fig. 6B.

20 Optical fiber **606** may incorporate a cylindrical diffuser as described in U.S. Pat. No. 6,013,053. Upon illumination by a light source as described above, the nanoparticles emit light in a diffuse non-directional fashion and also emit light at desired wavelengths or wavebands capable of activating the PDT drug or drugs present at the site. After treatment,

25 the matrix may either be removed or left in situ to biodegrade over time. The matrix is preferably composed of a biodegradable and bioresorbable material, such as polylactide. A preferred fiber, fabric or grid is formed of a biodegradable polymer as disclosed in the article by J. Middleton & A. Tipton, "Synthetic Biodegradable Polymers as Medical Devices," *Medical*

30 *Plastics & Biomaterials*, Mar./Apr. 1998, 30-39. Preferably, the matrix is flexible to ease placement within or on the patient.

As illustrated in Fig. 7A, optical fiber **606** may incorporate a total-internal reflection (TIR) lens **701** rather than a diffuser. The TIR lens in this instance receives light from the light source and is positioned in relation to the sheath **602** so that the lens focuses the light onto

5 nanoparticles **601**, which emit light to the treatment site **604**.

Fig. 7B depicts how a TIR lens **702** may be incorporated into the sheath **602** rather than the optical fiber. Light L_1 from optical fiber **606** illuminates nanoparticles **601**. The nanoparticles emit light L_2 , some of which is captured by TIR lens **702** attached to sheath **602**. Light L_2 is
10 focused by TIR lens **702** to form light L_3 , which illuminates target site **604**.

In this instance, it is preferred that the light source generating light L_1 is not a laser but is instead e.g. a light-emitting diode array or other source of non-coherent or non-laser light. Light L_1 will spread to
15 illuminate the quantum dots better. Thus, a non-coherent or non-laser light source is preferred in those instances where the surgeon prefers to have a light beam that diffuses after exiting the optical fiber.

Other examples of the invention are shown in FIG. 8A and 8B. An optical fiber **501** composed of a biocompatible matrix is shown.
20 Quantum dots **502** coat the external shell of the fiber or are embedded in the matrix. The fiber is inserted beneath a patient's skin **503** in proximity to the PDT treatment site. An external light source, such as an LED, a laser, a laser diode, or other non-coherent light source such as a incandescent lamp or halogen light, pumps light into the fiber during
25 treatment. In Fig. 8A, light L_3 from the quantum dot is spread over a wide area and is directed to diseased tissue **803**. In Fig. 8B, light L_1 from the quantum dot is captured by total internal reflection (TIR) lens **802** and is concentrated as light beam L_2 onto or into the tissue **802** to be treated. As an alternative or additionally, the quantum dots may coat the TIR lens
30 itself along portions of the TIR lens that form the optical pathway of the

lens, so that as much of the light generated by the dots fluorescing is directed by the lens.

Suitable internal reflection lenses are disclosed in the following U.S. patents, which are incorporated herein in their entirety as if fully put
5 forth below: U.S. Pat. No. 4,337,759, 5,404,869, 5,577,493, 5,613,769, 5,655,832, 5,676,453, 5,757,557, 5,806,955, 5,924,788, and 5,926,320. A total internal reflection lens is preferably configured so that it can be positioned within a patient to receive photodynamic therapy. For example, the TIR lens would be so configured if it met one
10 or more of the following criteria: the lens is sterile; the lens is sufficiently small to be implanted within a patient's body; the lens has a size suitable for illuminating the target tissue (e.g. blood; tumor; or organ); the lens is configured to receive light from an optical fiber that itself is adapted for use within a patient. A total internal reflection lens may capture some of
15 the light generated by light-emitting nanoparticles and emitted in a direction that would otherwise illuminate healthy areas if the TIR lens was not present. For example, light-emitting nanoparticles in a fiber optic sheath in sufficient proximity to the TIR lens to capture light that is emitted in a direction that would not illuminate the target site if the lens
20 was not present. The TIR lens then emits this captured light at the target site. A total internal reflection lens can thus increase the amount of nanoparticle-emitted light that illuminates diseased tissue or cells.

Instead of focusing light, a TIR lens may be used to diffuse light over a larger area but in the direction of the diseased tissue. If e.g. an
25 optical fiber or a light-emitting diode is placed at the focal point of the TIR lens rather than on the diffuse light-gathering side of the lens, the lens operates in reverse and diffuses the light to illuminate a broader area. Thus, light from the light source is spread over a larger area of the diseased tissue, or light emitted by nanoparticles embedded in a sheath is
30 spread over a larger area of the diseased tissue.

- In any of the embodiments above, light from the light source will also illuminate the target site. A site at which a PDT drug is activated by such light may thus be treated using a combination of PDT drugs, one or more which is activated by the light source directly and one or more
- 5 which is activated by light emitted by the nanoparticles.

7. Administration of light-emitting nanoparticles and PDT drug

- One embodiment of the present invention comprises administering a therapeutically effective amount of a PDT drug to a target area of a patient and also administering a sufficient quantity of light-emitting
- 10 nanoparticles to the target area to provide an illuminance or fluence of light that activates the PDT drug.

- PDT drugs are known in the art and are generally considered to be those drugs which absorb photons from a light source and transfer this energy to surrounding oxygen molecules. This in turn induces formation
- 15 of singlet oxygen and other highly-reactive free radical species, leading to a number of biological effects, including damage to proteins, nucleic acids, lipids, and other cellular components. A PDT drug thus destroys cancer cells, cells responsible for atherosclerosis, psoriasis, or macular degeneration, or other bodies responsible for bacterial or viral infections.
- 20 A PDT drug may instead be one which effects gene transcription.

- The nanoparticles are irradiated with light of a first wavelength. Photons of this light have energy that is greater than or equal to the bandgap energy of the material forming the nanoparticles, so that the nanoparticles absorb this energy and subsequently re-emit energy in the
- 25 form of light at a second wavelength. This light of a second wavelength is absorbed either by the PDT drug or by other nanoparticles than then re-emit this light at a third wavelength. The third wavelength is absorbed by a PDT drug or is absorbed by other nanoparticles and re-emitted at a fourth wavelength that is absorbed by a PDT drug.

a) Administration of PDT drug

- A PDT drug or photosensitizing agent is a chemical compound which homes to one or more types of selected target cells and, when contacted by radiation, absorbs the light, which results in impairment or destruction of the target cells. Virtually any chemical compound that homes to a selected target and absorbs light may be used in this invention. Preferably, the chemical compound is nontoxic to the animal to which it is administered or is capable of being formulated in a nontoxic composition. Preferably, the chemical compound in its photodegraded form is also nontoxic. A comprehensive listing of photosensitive chemicals may be found in Kreimer-Birnbaum, Sem. Hematol. 26:157-73, 1989. Photosensitive compounds include, but are not limited to: indocyanine green (ICG); methylene blue; toluidine blue; aminolevulinic acid (ALA); phthalocyanines; porphyrins; texaphyrins; bacteriochlorins; merocyanines, psoralens, benzoporphyrin derivatives (BPD) and porfimer sodium and pro-drugs such as α -aminolevulinic acid, which can produce drugs such as protoporphyrin. Also included are: chlorin compounds; purpurins; and any other agent that absorbs light in a range of 500 nm - 1100 nm. More specifically, chlorin and purpurin compounds contemplated in the present invention, include: mono-, di-, or polyamide aminodicarboxylic acid derivatives of cyclic or non-cyclic tetrapyrroles (see: Bommer et al., U.S. Patent Nos: 4,675,338 and 4,693,885); and alkyl ether derivatives of pyropheophorbide-a with N-substituted cyclic imides (purpurin-18 imides) (see: Pandey et al., WO 99/67249). Specifically, included are derivatives of mono-L-aspartyl chlorin e6 (NPe6); and any other agent that absorbs light in a range of 500 nm - 1100 nm.

- Other examples of PDT drugs include but are not limited to photosensitive compounds such as pheophorbides and pyropheophorbides. Porfimer sodium or omeprazole can be used to relieve symptoms of esophageal cancer. BPD verteporfin may be used for

skin carcinoma, cutaneous lesions where cancer has metastasized to the skin, and chronic stable plaque psoriasis. BPD verteporfin is activated by red light having a wavelength of 690 nm and also by ultraviolet A light having a wavelength of 290-320 nm. Hematoporphyrin derivatives and especially dihematoporphyrin ether, which has a peak absorption at a wavelength of 405 nm and a low absorption peak at 630 nm, are also useful. PDT drugs include those that treat cancer of the bronchi, pharynx, oral cavity, bladder, eyes, and the uterus. Other examples of PDT drugs include Pharmacyclics' ANTRIN[®], which may be used for photoangioplasty treatment of patients with peripheral vascular disease to remove arterial plaque; boronated porphyrin, being tested to treat brain cancer; Scotia QuantaNova's drug FOSCAN[®], being tested to treat head and neck cancer; and QLT PhotoTherapeutics' PHOTOFRIN[®] (a precancerous condition of the esophagus).

A PDT drug often absorbs light at multiple wavelengths that are typically within a bandwidth of about 300 nm. Also, a number of drugs absorb light at a wavelength that is more difficult to deliver to the drug using current lasers or other light sources such as LEDs. Dihematoporphyrin ether absorbs light having a wavelength of 405 nm most effectively, yet it is not practical to deliver this shorter wavelength of light into the body because of the short penetration depth of light of this wavelength (on the order of a millimeter or less). Consequently, dihematoporphyrin ether is instead activated using light having a wavelength at which the drug absorbs more energy. Use of light-emitting nanoparticles that absorb light having a wavelength greater than 405 nm (e.g. 805 nm) and emit light at 405 nm is one option when practicing the invention. The 405 nm light emitted by the nanoparticles activates the dihematoporphyrin ether directly. Another option for this drug is to have both nanoparticles that emit light at 630 nm as well as nanoparticles that emit light at 405 nm together as part of the fluid that is administered to a

patient and use a different wavelength or wavelengths of light from the light source to cause the nanoparticles to fluoresce.

A therapeutically effective amount of a PDT drug is administered to the patient. The drug is therefore present in the target site in an amount
5 that produces a sufficient quantity of active species (such as singlet oxygen or other reactive species) to effect the particular therapy being administered. Only a fraction of the radicals or other reactive species generated by the drug damage critical cell sites to cause cell death or begin gene transcription or provide another beneficial effect.
10 Consequently, the quantity of PDT drug is selected to assure that the beneficial effect of the drug is obtained. A typical dosage is between about 0.05 and 5 mg of PDT drug per kilogram of body weight.

Administration of PDT drugs to a patient may be systemic, by ingestion or injection; regional (e.g. to the gastrointestinal, hepatic, or
15 renal systems, for example, or to anatomic regions such as the abdomen, lumbar spine, arm or leg regions), by intravenous injection or topical application; or by affinity targeting, by applying the compound to a specific treatment site on or in the patient's body via topical administration or injection into the target site, or by ingestion with a
20 feeding tube. These and other possible methods of administration of PDT drugs are known to those skilled in the art and are included in the present invention.

b) Administration of nanoparticles

A therapeutically effective amount of light emitting nanoparticles is
25 also administered to the patient. Activating a PDT drug to effect a particular therapy (e.g. tumor or psoriasis treatment or gene transcription) requires a certain amount of light to reach the PDT drug. A quantity of light insufficient to effect therapy may cause some drug molecules to form reactive species such as single oxygen, but an insufficient number
30 of the reactive species are generated to reduce tumor size or arrest tumor growth, for instance. A therapeutically-effective amount of nanoparticles

is therefore a quantity that generates a sufficient amount of light to the PDT drug to produce the desired therapy, either alone or in combination with light supplied by the light source used to excite the nanoparticles to emit light. The amount of light and therefore the number of nanoparticles

5 is a function of many factors, which include: the distance the nanoparticles are from the drug to be activated; the type and density of tissue, if any, in the optical path between the drug and the nanoparticles; the number of photons to which the drug is exposed, the wavelength of light used to activate the drug; the extinction coefficient of the PDT drug;

10 the amount of PDT drug present; the length of time that the PDT drug is exposed to activating light; the oxygen concentration in the tissue neighboring the PDT drug (which affects the quantum yield of radicals); the fraction of radicals that actually contact the target site; and the optical penetration depth of the light that activates the PDT drug.

15 A sufficient quantity of nanoparticles emitting the activating wavelength is therefore provided to the target site so that these nanoparticles are sufficiently close to the PDT drug to activate it. As noted previously, light of shorter wavelengths is readily absorbed by tissue. Consequently, a PDT drug that absorbs short wavelength light

20 requires the light source to be very close to the PDT drug. Otherwise, tissue attenuates the light before it can activate the drug. Light emitting nanoparticles can be positioned sufficiently closely to PDT drug molecules to overcome the problem of tissue absorbing the light which activates the PDT drug.

25 A sufficient quantity of light-emitting nanoparticles close to the PDT drug can be provided in a number of ways. One way is to administer a sufficiently high number of nanoparticles to the patient systematically, regionally, or locally so that the desired concentration of nanoparticles is achieved in the vicinity of the PDT drug. Another way is

30 to attach one or more delivery moieties to the nanoparticles that are specific to the target site area, so that the nanoparticles localize in the

treatment area. The delivery moieties may bind specifically to a material produced by or found in the target area, or the delivery moieties may bind to the PDT drug. A third way to place nanoparticles within sufficient proximity to the PDT drug is to attach the nanoparticles to the drug either

5 covalently or through an associating group, as described herein.

Thus, the light-emitting nanoparticles may be unmodified, may be solubilized, or may be attached to a delivery moiety as described above, thus targeting the nanoparticles to the treatment site. Nanoparticles attached to a delivery moiety have a wider time range over which they

10 may be administered. Since often the delivery moiety is site-specific, nanoparticles having a delivery moiety are retained at the target site longer than nanoparticles not having a delivery moiety. Consequently, nanoparticles having a delivery moiety can be administered sooner than nanoparticles not having a delivery moiety. Further, the affinity that a

15 delivery moiety has for the target site may allow such nanoparticles to be accumulated at a target site within a shorter period of time than nanoparticles without such groups require to accumulate at the site.

In one form of systemic PDT, the photosensitizing agent (also referred to as "PDT drug") is injected into the blood- stream at time t_0 .

20 Cells within the body absorb the PDT drug. However, the PDT drug resides in disease cells (such as cancer cells) for a longer period of time than the drug resides in healthy or normal cells. Consequently, light administration is timed to occur at a time t_2 such that a sufficient amount of the PDT drug has dissipated to low levels in or near healthy cells. The

25 PDT drug is also administered to the patient in an amount that results in a sufficient level of PDT drug in disease cells when the drug has dissipated sufficiently from healthy or normal cells such that PDT can commence without substantial harm to a significant number of healthy or normal cells.

30 A therapeutically effective quantity of nanoparticles may also be administered systematically to the patient by injecting them into the

blood-stream. The nanoparticles are injected or otherwise systematically administered at a time t_1 to provide a sufficiently high concentration in sufficiently close proximity to the PDT drug to activate the drug at the appropriate time. Typically, the time t_1 occurs at or after time t_0 , and usually the time t_1 occurs before t_2 . Thus, the PDT drug and the nanoparticles may be administered simultaneously and allowed time to reach the treatment site before irradiating the site, or the nanoparticles may be administered after the drug is administered but before the time that the target site is irradiated.

The light-emitting nanoparticles may also be administered to the patient as light from the light source irradiates the target site, especially where the nanoparticles are injected locally into the patient or where the nanoparticles have delivery moieties that result in a sufficient number of the nanoparticles accumulating in the target area and activating the PDT drug to effect treatment. An area of the body will not receive photodynamic therapy so long as that area is not illuminated with light of a wavelength that causes the nanoparticles to fluoresce or does not contain a sufficiently high concentration of nanoparticles to cause significant amounts of PDT drug activation.

In systematic PDT drug administration, the time t_2 at which light is administered is typically one to three days after time t_0 , the time at which the PDT drug is administered systematically, where the drug is used to treat cancers of the esophagus, bronchial tubes, cervix, pancreas, or colon for instance. The time t_1 at which nanoparticles are administered in these instances is typically between 1 hour and 72 hours after the time at which the PDT drug is administered.

If the PDT drug is one used to treat wet macular degeneration, the time t_2 at which time is administered is typically several minutes after the photosensitive drug has been administered intravenously. Nanoparticles may be administered at any time t_1 before time t_2 so long as there is sufficient time to build a sufficient concentration of the nanoparticles in

close proximity to the photoactive drug at the site to be treated. The rapidly-growing, new blood vessels which grow beneath the retina and bleed are treated, whereas the normal vessels are essentially undisturbed.

Multiple PDT drugs may be administered and/or activated simultaneously or sequentially. Consequently, two or more PDT drugs may be administered separately but allowed sufficient time to accumulate at a treatment site, then the drugs are activated simultaneously when the light source illuminates the target area and when sufficient light-emitting nanoparticles are present. Further, the drugs may be activated separately by supplying a mixture of nanoparticles (one type of nanoparticle that emits wavelength λ_1 and activates drug number 1, a second type that emits wavelength λ_2 and activates drug number 2, and so forth) and by irradiating the area with activating light from a light source that supplies a first wavelength of actinic light sufficient to cause the nanoparticles to emit λ_1 , then irradiating the area with activating light that supplies a second wavelength sufficient to cause the nanoparticles to emit λ_2 and so forth.

c) Light activation

As illustrated in Fig. 4, as or after a sufficient number of nanoparticles accumulate at the treatment site, a light source 401 is introduced into a treatment site 402 beneath the skin 403, or a light source 404 is applied to the patient's skin externally to generate light waves 405 that irradiate light-emitting nanoparticles 201 attached to site-specific antigens 202. The light source may be any suitable source which provides light that is absorbed by the nanoparticles structure and which optionally penetrates tissue. Light sources that may be used in accordance with the present intention include but are not limited to lasers and laser diodes, light emitting diodes (LEDs) formed of semiconductor or organic materials, electroluminescent light sources, incandescent light sources, cold cathode fluorescent light sources, and other light sources emitting light of a wavelength capable of causing the light-emitting

nanoparticles to fluoresce. Tissue-penetrating light sources typically emit one or more wavelengths within the 350-1100 nm waveband, and preferably emit one or more wavelengths within the 600-100 nm waveband.

5 Light may be delivered subcutaneously by coupling the light to a fiber optic and directing it through e.g. a bronchoscope into the lungs for the treatment of lung cancer, through an endoscope into the esophagus for the treatment of esophageal cancer, or through a catheter into or near the tumor at another location within the body. One preferred light source
10 is a portable light source that a patient may wear continuously through the treatment period, as disclosed in U.S. Ser. No. 09/232,129, "Patient Portable Device For Photodynamic Therapy," James Chen et al., filed Jan. 15, 1999 (also disclosed in PCT/US00/00805, "Patient Portable Device For Photodynamic Therapy," James Chen et al., filed Jan 14, 2000).

15 A preferred internal light source is a laser coupled to an optical fiber. The fiber may have a diffusing tip attached, such as a cylindrically-shaped or spherically-shaped diffuser. A preferred light source is an external LED array focused with a totally internally reflecting lens, such as that disclosed in WO 98/33251 and WO 98/20936.

20 Wavelengths and wavebands longer than 600 nm are preferred for an external light source, because they are capable of penetrating tissue fairly deeply. Light at a wavelength of 630 nm effectively penetrates approximately 3 cm into tissue before the optical energy has been dissipated. An external light source is useful for treating psoriasis, skin
25 carcinomas, and other disorders in which the target site is located within approximately 3 cm of the skin surface. Other wavelengths include those at which two-photon activation occurs (e.g. between 750 and 1240 nm, and especially about 800 nm).

A light source typically produces a range of wavelengths
30 ("waveband"). A laser produces a narrow waveband of light, whereas an LED array produces a broad waveband or multiple wavebands of light.

The waveband of the light source is selected to stimulate emission from the light-emitting nanoparticles selected for the particular therapy to be administered.

- It is often difficult to provide light of wavelengths suitable to
- 5 activate one or more drugs at multiple wavelengths. As noted above, a laser produces wavelengths within a narrow waveband. Consequently, multiple lasers are needed to activate a drug or multiple drugs at multiple wavelengths. Further, LEDs are not yet commercially available that emit in different waveband ranges. LEDs that emit in the blue light region are
 - 10 currently being commercialized, but LEDs having shorter wavelengths in the blue-violet, violet, and ultraviolet wavelength range are not readily available. The use of light-emitting nanoparticles enables a surgeon to irradiate a treatment site with narrow-band light or light having multiple wavebands and convert that light into light of other wavelengths and
 - 15 wavebands. It is thus possible to convert light of a narrow waveband into broadband emission or into light emission of multiple desired wavebands specific to the drug or drugs selected for use in PDT by selecting the light-emitting nanoparticles accordingly.

sub 07
20 The activating light source emits light that the nanoparticles absorb. Consequently, if the nanoparticles all absorb light at about the same wavelength, a narrow-band light source such as a laser can be used. Or, if the nanoparticles absorb light at different wavelengths, a broader-band light source may be used such as an LED array. Light-emitting nanoparticles may themselves be a light source for other light-emitting

- 25 nanoparticles as described previously in order to allow a light source to be used that produces light of a wavelength that is absorbed by some of the nanoparticles but not all of them. In this case, the nanoparticles acting as a light source for other nanoparticles absorb the actinic radiation from the light source, then emit radiation at a second wavelength that at least
- 30 some of the other nanoparticles absorb, causing them to fluoresce. In another preferred embodiment of the invention, a narrow-band light

source such as a laser is used to activate a mixture of nanoparticles whose emission provides a broad band of light.

In one embodiment of the invention, the wavelength range emitted from the external or internal light source is selected such that it does not activate the PDT drug directly. For example, the light source can be selected to produce light having a wavelength within the range of about 600 or 800 nm to about 1100 nm, which does not activate some presently-available PDT drugs effectively. The light source activates the nanoparticles, and the nanoparticles in turn re-emit the light as fluorescence at a wavelength or waveband that closely matches the absorption band(s) of the PDT drug (e.g. at a wavelength of 405 nm and 630 nm for dihematoporphyrin ether). The drug is thus activated by way of the photoluminescence from the nanoparticles. Excitation of the drug in turn leads to formation of singlet oxygen and other reactive species and result in cell death. Combinations of different types of PDT drugs and/or different types of nanoparticles may be used simultaneously to achieve desired effects for a particular condition or treatment.

8. Other preferred therapies

One preferred therapy involves administering light-emitting nanoparticles and PDT Drug that are bound to one another either directly or through a backbone polymer and illuminating a target site in a patient to cause the nanoparticles to fluoresce. A sufficient number of light-emitting nanoparticles are bound to the PDT drug so that they are in the vicinity of the drug during use and illuminate the drug, thus producing singlet oxygen. The nanoparticles are illuminated using either a light source external to the patient or one internal to the patient.

Another preferred therapy involves administering a PDT drug and light-emitting nanoparticles that are attached to one or more types of delivery moieties, and illuminating the target site with light that causes the nanoparticles to fluoresce after the nanoparticles are given sufficient time to associate or bind with the target site. The target site has proteins

or other molecules, cells, tissue, or other materials for which the delivery moieties have an affinity, as described previously.

In one therapy, the nanoparticles are selected to provide a desired intensity of light at a given wavelength. A mixture of nanoparticles as
5 described above (e.g. mixtures of different sizes and/or compositions; mixtures of quantum dots, nanocrystals, and/or quantum rods) is formed to provide the desired light intensities at various wavelengths, and the mixture is administered to the patient to activate either a single PDT drug that absorbs light at multiple wavelengths or a mixture of PDT drugs, any
10 of which absorbs light at a single wavelength or multiple wavelengths.

In another preferred embodiment of the invention, a mixture of nanoparticles is administered that provides multiple wavebands of light. The wavebands are selected to provide light of a wavelength that is absorbed by a PDT drug that has also been administered to the patient.
15 The mixture is also optionally formed to provide different light intensities for the different wavebands present, so that the intensity varies as a function of the absorption peak for the PDT drug or drugs used in the therapy. Consequently, the particle size and type (e.g. quantum dots, nanocrystals, and/or quantum rods) of the nanoparticles are selected to
20 provide the desired wavebands and waveband intensities.

Any of the components, compositions, drugs, and tools discussed herein as being placed within a patient are preferably sterile or otherwise suitable for placement within the patient. Thus, any optic fiber, TIR lens, nanoparticles, and PDT drug to be placed within a patient are preferably
25 sterile. Further, there may be packaged combinations of these materials. For example, an optic fiber may be packaged with nanoparticles, where the nanoparticles are separate from the optic fiber or are unitary with (e.g. embedded in, fixed upon, or otherwise part of) the optic fiber. Nanoparticles may be packed with a PDT drug or combination of drugs
30 that is activated at the wavelength or waveband of light emitted by the nanoparticles. Further, the PDT drug may be a combination of drugs that

activate at different wavelengths (such as at wavelengths emitted by the nanoparticles and optionally at the wavelength emitted by the light source). Any of the components or kits of components may also contain a set of instructions for use of the components as are utilized in the

5 medical field to instruct a surgeon on use of the component or components. Thus, for example, a PDT drug may have instructions on dosage and administration of the drug. An optic fiber may have instructions for insertion and/or use within a patient. Nanoparticles may be accompanied by instructions to use only with a particular PDT drug.

10 The invention thus provides new or improved tools, compositions, and methods associated with treatment of disease, and the invention is intended to cover all such new or improved tools, and methods disclosed herein.

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